Introduction: While tendon injury is common, there has been minimal progress in developing medical therapies to improve healing or to decrease the long-term consequences of the repair process. Among the most serious consequences is the development of scar tissue that results in adhesions. Adhesions are particularly problematic in intra-synovial tendon healing since they reduce tendon gliding efficiency and motion. Anabolic therapies that would increase the rate of healing and permit earlier return to full function and/or reduce adhesion formation would be a major advance.

PTH 1-34, a biologically active fragment of parathyroid hormone, is approved for clinical use as an anabolic agent to increase bone formation in patients with osteoporosis. Preclinical studies demonstrate that PTH enhances fracture healing and works as an anabolic agent by activating stem cell populations in the injury and repair environment. Tendon repair is analogous to fracture healing in its dependence on proliferation and differentiation mesenchymal stem cells, matrix formation, and tissue remodeling. The current study was conducted to determine whether PTH has anabolic effects on intra-synovial tendon healing. We tested the hypothesis that PTH enhances tendon-to-tendon healing using both a mouse model of primary flexor digitorum longus (FDL) tendon repair of a fully transected tendon (1) and the collagen gel contraction assay in which cultured tenocytes remodel their pericellular extracellular matrix (2).

Methods: Mouse model of tendon healing: All animals were cared for in accordance with an animal use and care protocol approved by the University Committee on Animal Research (UCAR). The FDL tendon was transected and immediately repaired using a modified Kessler technique in 6-8 week old male mice (1). The tendon was also released at the myotendinous junction to prevent early transmission of active forces against the tendon repair. Mice typically regain active flexion between 21 and 28 days in this model. Ninety seven mice underwent repair alone and ninety seven mice received daily subcutaneous injections of 40 μg/kg of PTH 1-34 (Sigma-Aldrich, St. Louis, MO) beginning on the date of surgery. Limbs were harvested on post-operative days 3, 7, 14, 21 and 28 days for histological analysis (N=4 repairs per time point), immunohistochemistry (IHC) (N=4 repairs per time point) and RNA extraction for real-time RT-PCR (N=5 repairs per time point). Additionally, limbs were harvested at 0, 14, 21, and 28 days post-surgery for biomechanical strength testing (N=8 repairs per time point).

In vitro model of tendon healing: FDL tendons were isolated from 7-month old male mice. Tendon cells expanded in cell culture were mixed with a collagen I solution at a density of 7x10⁵ cells/ml. The cell-seeded collagen was cast into custom-made silicone constructs, and fresh media (MEM α supplemented with 10% FBS and 1% Pen Strep) with or without 100 nM PTH 1-34 (Sigma-Aldrich, St. Louis, MO) was added to the wells. Gel area contraction was measured for a total sample size of 6 per treatment per time point.

Real-time RT-PCR and biomechanical and contraction data were analyzed using a two-way analysis of variance (ANOVA) with Bonferroni’s multiple comparisons at the α = 0.05 significance level.

Results: Histology: On post-operative day 3, PTH treated mice displayed extensive recruitment of inflammatory cells to the injury site while minimal cellularity was observed in their vehicle treated counterparts. On day 7, an external callus of cells from the epitendon had bridged the tendon ends in the PTH treated mice, while less fibroblastic granulation tissue had filled in the repair site in the vehicle treated mice and external callus was not yet present. At 14 and 21 days post-repair, the external callus had grown larger and was beginning to remodel and replace the adjacent tendon ends in the PTH treated mice. By 28 days, the PTH treated mice displayed progressive tendon remodeling with dense collagen fiber organization parallel to the tendon axis. Vehicle treated mice at 14, 21 and 28 days post-repair had smaller callus sizes, more native tendon, and less collagen fiber organization compared to PTH treated mice.

Gene and protein expression: Real-time PCR analysis showed a 2-fold increase in col1a1 gene expression in PTH treated mice at 14 and 21 days post-repair (Fig. 1A; p<0.05). Col1a1 and fn1 gene expression were 1.5- and 2-fold greater in PTH treated mice at 21 days post-repair, respectively (Fig. 1B and C; p<0.05). PTH1R gene expression was 2- and 2.5-fold greater in PTH treated mice at 14 (p<0.05) and 21 days (p<0.001) post-repair (Fig. 1D). IHC confirmed the findings, showing that staining of type I and III collagen and PTH1 receptor protein was greater in PTH treated mice compared to vehicle treated mice at all times.

Biomechanical testing: Non-destructive metatarsophalangeal (MTP) joint flexion testing showed that the MTP joint range of motion (ROM) was 1.5- to 2-fold lower for PTH compared to WT mice throughout the duration of the experiment (Fig. 2A; p<0.001). Similarly, the gliding coefficient, a measure of resistance to joint ROM, was 2- to 3.5-fold higher in PTH mice (Fig. 2B; p<0.05). Maximum tensile strength testing showed significantly greater tensile strength for PTH mice at 14 days post-repair (Fig. 2C; p<0.01).

Gel contraction: Contraction analysis showed that gels treated with PTH 1-34 contracted to an average of 61% of their initial area after 48 hours, whereas those treated with control media contracted to only 86% of their original area (Fig. 2D; p<0.001).
Discussion: PTH is an important anabolic agent for the treatment of osteoporosis and is being investigated as an agent to enhance injury and repair processes. These experiments demonstrate that PTH has an anabolic effect on murine intra-synovial tendon healing. Histology demonstrated earlier and more robust accumulation of reparative tissue. This was associated with an increased expression of genes involved in tissue repair, including type I and III collagen and fibronectin. IHC showed similar increased expression of the collagens. Interestingly, we observed that treatment with PTH increased gene and protein expression of PTHR1, the cell surface receptor for PTH. This suggests that activation of the PTH signaling pathway in soft tissue repair further increases receptor mediated cell and tissue responses.

As tendon function involves motion in response to tensile forces, biomechanical testing is a direct measure of tendon healing. We performed a biomechanical test of digit motion in situ in harvested limbs to measure digit ROM and determine the adhesion coefficient. Although PTH increased the strength of tendon repair, it increased adhesions and resulted in a decrease in the ROM. This finding was supported by in vitro analysis that showed increased gel contraction in PTH-treated cultured tenocytes.

Significance: Altogether our findings demonstrate that PTH exerts an anabolic response on tendon healing and increases the deposition of repair tissue and enhances the mechanical strength of repair. However, this is associated with an increase in the formation of adhesions. The data suggest that PTH may be an effective therapy to enhance healing. Because of the effect on adhesions, PTH might be a more useful therapy for the repair of extra-synovial tendons with limited gliding function, such as the rotator cuff and patellar tendon.

Acknowledgments: This study was funded by the University of Rochester CTSA award number TL1 TR000096.
